

Impaired Virus Control and Severe CD8⁺ T-Cell-Mediated Immunopathology in Chimeric Mice Deficient in Gamma Interferon Receptor Expression on both Parenchymal and Hematopoietic Cells

Pernille Henriksen, Christina Bartholdy, Jan Pravsgaard Christensen,
and Allan Randrup Thomsen*

Institute of Medical Microbiology and Immunology, University of Copenhagen, Copenhagen, Denmark

Received 10 January 2005/Accepted 15 April 2005

Bone marrow chimeras were used to determine the cellular target(s) for the antiviral activity of gamma interferon (IFN- γ). By transfusing such mice with high numbers of naive virus-specific CD8⁺ T cells, a system was created in which the majority of virus-specific CD8⁺ T cells would be capable of responding to IFN- γ , but expression of the relevant receptor on non-T cells could be experimentally controlled. Only when the IFN- γ receptor is absent on both radioresistant parenchymal and bone marrow-derived cells will chimeric mice challenged with a highly invasive, noncytolytic virus completely lack the ability to control the infection and develop severe wasting disease. Further, the study shows that IFN- γ receptor expression on parenchymal cells in the viscera is more important for virus control than IFN- γ receptor expression on bone marrow-derived cells.

CD8⁺ T cells are central effectors in antiviral immunity. They exert their effector activity by contact-dependent lysis of infected cells (11, 26, 30) and/or secretion of cytokines such as tumor necrosis factor alpha and gamma interferon (IFN- γ) (10, 20, 21). Although often central in antiviral immunity, the exact role of IFN- γ is still unclear.

While certain viral infections may be controlled in the absence of IFN- γ (8, 13–16, 22), the clearance of many other viruses critically depends on the activity of this cytokine (7, 9, 13, 14, 23, 24). With regard to lymphocytic choriomeningitis virus (LCMV), which is a commonly used viral model, early studies indicated that IFN- γ played a relatively minor role in the clearance of an acute infection (9, 16, 25, 28, 29). However, more recent studies by our group revealed that IFN- γ is required to resolve this infection. Thus, IFN- γ -deficient (IFN- γ ^{−/−}) mice were unable to clear a slowly replicating strain of LCMV (Armstrong) and became chronically infected (1), whereas IFN- γ ^{−/−} mice infected with a highly invasive strain (LCMV Traub) succumbed due to CD8⁺ T-cell-mediated wasting disease accompanied by total impairment of virus clearance (17).

The pathology seems to be the result of an unfortunate imbalance between virus replication in the major internal organs and the host's futile attempt to eliminate infected cells (17).

Wasting disease can be prevented if the T-cell responsiveness of the host is increased. This can be achieved by adoptive transfer of naïve virus-specific, T-cell receptor transgenic

(TCR-tg) CD8⁺ T cells into the IFN- γ ^{−/−} mice before virus challenge (17).

Regarding the mechanism underlying the critical role of IFN- γ , no unequivocal explanation has been presented so far. One group has suggested that IFN- γ may be involved in generation of cytotoxic T lymphocytes (CTLs), since treatment with antibodies against IFN- γ was associated with decreased levels of CTLs and complete impairment of virus clearance of a highly invasive virus strain (27, 32). Furthermore, virus persistence is always associated with some measure of T-cell dysfunction (anergy/deletion) (18). However, there is no absolute requirement for IFN- γ in the generation of CTLs. Thus, if IFN- γ is not essential for virus control, as in the case of the murine vesicular stomatitis virus infection, the antiviral CD8⁺ T-cell response is unimpaired in IFN- γ (5)- and IFN- γ receptor-deficient (IFN- γ R^{−/−}) mice (Christensen et al., unpublished observation) with regard to both cell numbers and functionality (cytokine production). Moreover, the primary virus-specific CD8⁺ T-cell response is not reduced in IFN- γ R^{−/−} mice infected with slowly replicating strains of LCMV (18). Thus, rather than being the primary phenomenon, T-cell dysfunction is more likely to be the result of overwhelming infection and high-dose immune paralysis in the absence of IFN- γ -dependent virus control (5, 12, 18, 31).

In the present study, we wanted to investigate whether IFN- γ affected the outcome through activities on non-T cells. This question was addressed by studying the outcome of infection in mice lacking IFN- γ R expression on parenchymal and/or bone marrow-derived cells. By transfusing such mice with high numbers of naive wild-type (WT) T cells (from TCR transgenic mice) prior to infection, we created a system where the donor-derived CD8⁺ T cells would completely dominate (3; our own unpublished observation). As a consequence the majority of CD8⁺ T cells would therefore be capable of both producing

* Corresponding author. Mailing address: Institute of Medical Microbiology and Immunology, The Panum Institute, 3C Blegdamsvej, DK-2200 Copenhagen N, Denmark. Phone: 45 35327871. Fax: 45 35327891. E-mail: a.r.thomsen@immi.ku.dk.

and responding to IFN- γ , but the response in non-T cells could be experimentally controlled.

In order to validate the use of IFN- γ R $^{-/-}$ mice in this setup, we first compared the outcomes of systemic infection with a highly invasive strain of LCMV in IFN- γ $^{-/-}$ and IFN- γ R $^{-/-}$ mice (6, 9). These mice and WT C57BL/6 mice were inoculated intravenously (i.v.) with 1,000 50% lethal doses (LD₅₀) of LCMV Traub. The mice were weighed daily, and on day 8 postinfection (p.i.), some of the mice were sacrificed for evaluation of viral titers in spleen and liver using an immune focus plaque assay (2, 4).

Mice deficient in IFN- γ begin to die from the infection around day 8 to 9 p.i. (17). We found that IFN- γ R $^{-/-}$ mice were at least as susceptible as IFN- γ $^{-/-}$ mice (Fig. 1A and C), and in both strains the loss in body weight was negatively correlated with the capacity to reduce the virus load in spleen and liver (Fig. 2).

As shown previously IFN- γ $^{-/-}$ mice can be rescued if T-cell responsiveness is raised by adoptively transferring naive, virus-specific TCR-tg CD8 $^{+}$ T cells to the mice prior to infection. In order to clarify whether this effect is merely the result of a numerical increase in the number of effector T cells, we compared the outcomes of infection in IFN- γ $^{-/-}$ and IFN- γ R $^{-/-}$ mice, which had received 3×10^6 donor cells from TCR-tg mice (line 318) (19) 1 day prior to virus challenge. In agreement with a previous study by our group, IFN- γ $^{-/-}$ mice survived the infection with few symptoms (Fig. 1B and D), and this correlated with rapid virus control in the recipients (Fig. 2) (17). In contrast, high virus titers were found in livers and spleens of IFN- γ R $^{-/-}$ mice, which also developed severe wasting disease (Fig. 1B and 2). From this observation we conclude that it is not simply the increased number of effector cells that rescue mice with a defective IFN- γ response. Furthermore, with the number of TCR-tg CD8 $^{+}$ T cells transferred, donor-derived WT (IFN- γ R $^{+}$) CD8 $^{+}$ T cells would totally dominate the virus-specific response (3; own unpublished observation). Consequently, we conclude that IFN- γ needs to act on one or more host-derived non-T-cell populations.

To better define the cellular target(s) for the activity of IFN- γ in vivo, syngeneic and allogeneic bone marrow chimeras were made using IFN- γ R $^{-/-}$ and WT mice. Lethally irradiated (900 rads) mice were transplanted intravenously (i.v.) with 20×10^6 femur cells from allo- or syngeneic donors. After 8 weeks each mouse received 3×10^6 donor cells from TCR-tg mice i.v., and 1 day later the mice were infected with 1,000 LD₅₀ LCMV Traub i.v. Mice were weighed daily until day 10 p.i., when they were sacrificed and the organs were removed for analysis of virus loads.

Mice lacking IFN- γ receptor expression on both radioresistant, parenchymal cells and nonspecific bone marrow-derived cells developed severe wasting disease (Fig. 3A) and had high virus loads in livers and spleens (Fig. 3B). None of the other chimeras suffered to the same extent from weight loss or impairment of virus clearance. This observation indicates that an IFN- γ -induced response must be absent both in the viscera as such and in bone marrow-derived cells for severe impairment of virus clearance and for CD8 $^{+}$ T-cell-mediated wasting disease to become lethal. Furthermore, with regard to antiviral protection, an intact IFN- γ response in the parenchymal cells is more important than in the bone marrow-derived cells.

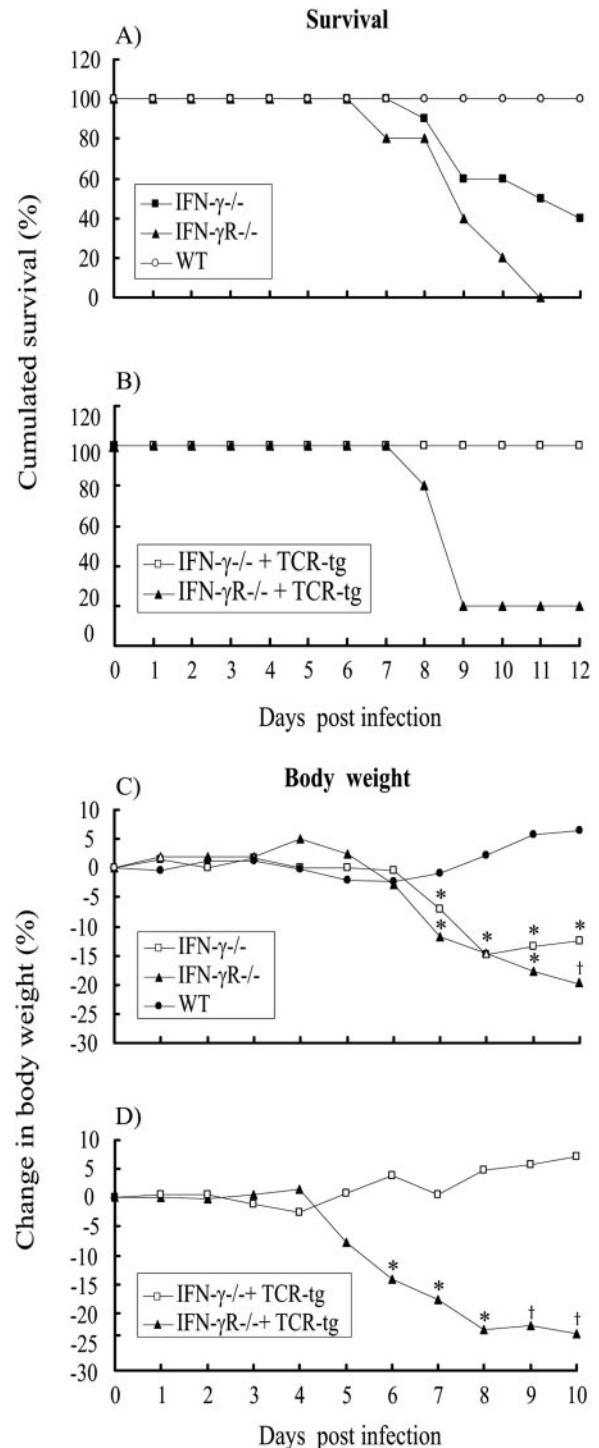


FIG. 1. Adoptive transfer of naive TCR-tg cells rescues IFN- γ $^{-/-}$ mice, but not IFN- γ R $^{-/-}$ mice. IFN- γ $^{-/-}$, IFN- γ R $^{-/-}$, and WT mice were infected i.v. with 10^3 LD₅₀ LCMV Traub. Survival (A) and virus-induced weight loss (C) were followed until day 12 postinfection (5 to 10 mice/group). One day prior to virus challenge, part of the IFN- γ $^{-/-}$ and IFN- γ R $^{-/-}$ mice received 3×10^6 donor cells from TCR-tg mice i.v. Survival (B) and virus-induced weight loss (D) were followed (5 mice/group). Changes in body weights are presented as median percentages of 5 to 10 mice/group. *, statistically significant differences at $P < 0.05$ (Mann-Whitney U test); †, too few observations for statistical analysis.

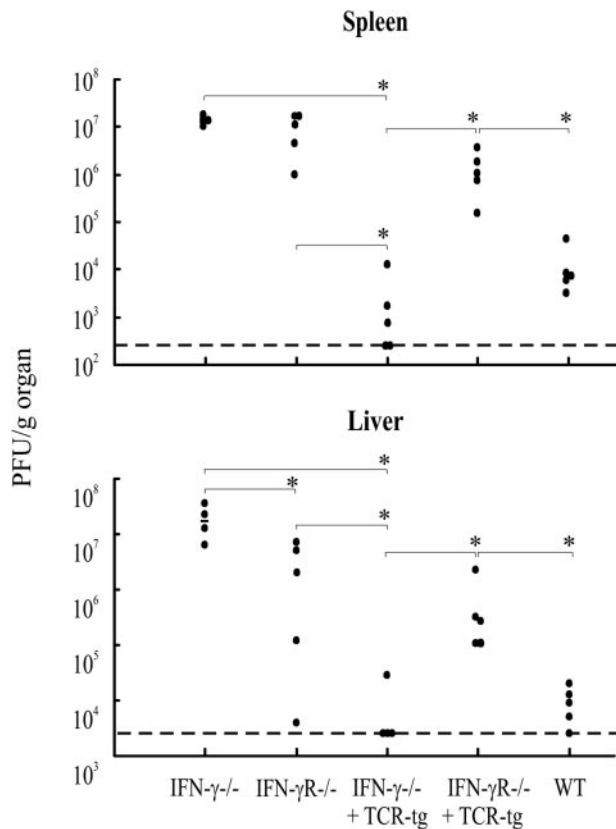


FIG. 2. Adoptive transfer of naïve TCR-tg cells reestablishes virus control in IFN- γ ^{-/-} mice, but not in IFN- γ R^{-/-} mice. IFN- γ ^{-/-}, IFN- γ R^{-/-}, and WT mice were infected intravenously with 10^3 LD₅₀ LCMV Traub. One day prior to virus challenge, part of the IFN- γ ^{-/-} and IFN- γ R^{-/-} mice received 3×10^6 cells from TCR-tg mice i.v. The mice were sacrificed day 8 postinfection, and virus titers in spleens and livers were determined. Points represent individual mice. Dashed lines indicate the detection limit. *, statistically significant differences at $P < 0.05$ (Mann-Whitney U test).

Thus, chimeras having a wild-type hematopoietic compartment but a disrupted IFN- γ response in the parenchymal compartment suffered from more severe wasting and had higher virus titers in their organs (Fig. 3A and B) than chimeras which had a wild-type parenchymal compartment but lacked IFN- γ receptor expression on the bone marrow-derived cells. The last group cleared virus with the same efficiency and exhibited the same pattern of a mild, transient weight loss as wild-type mice reconstituted with wild-type femur cells.

The fact that it is most important to have an intact IFN- γ response in parenchymal cells indicates that IFN- γ exerts the most essential part of its antiviral role in the viscera by rendering uninfected parenchymal cells refractory to infection, thereby limiting the virus spread.

Additionally, the results strongly suggest that expression of the IFN- γ receptor is not essential on the antigen-presenting cells. Nor is the activation of monocytes/macrophages through this receptor critical during T-cell-mediated virus clearance, unless IFN- γ also does not inhibit virus replication in the parenchymal cells. The latter observation could reflect that if virus replication in the parenchymal cells is slowed down, direct cytolysis may suffice as an effector mechanism for the T

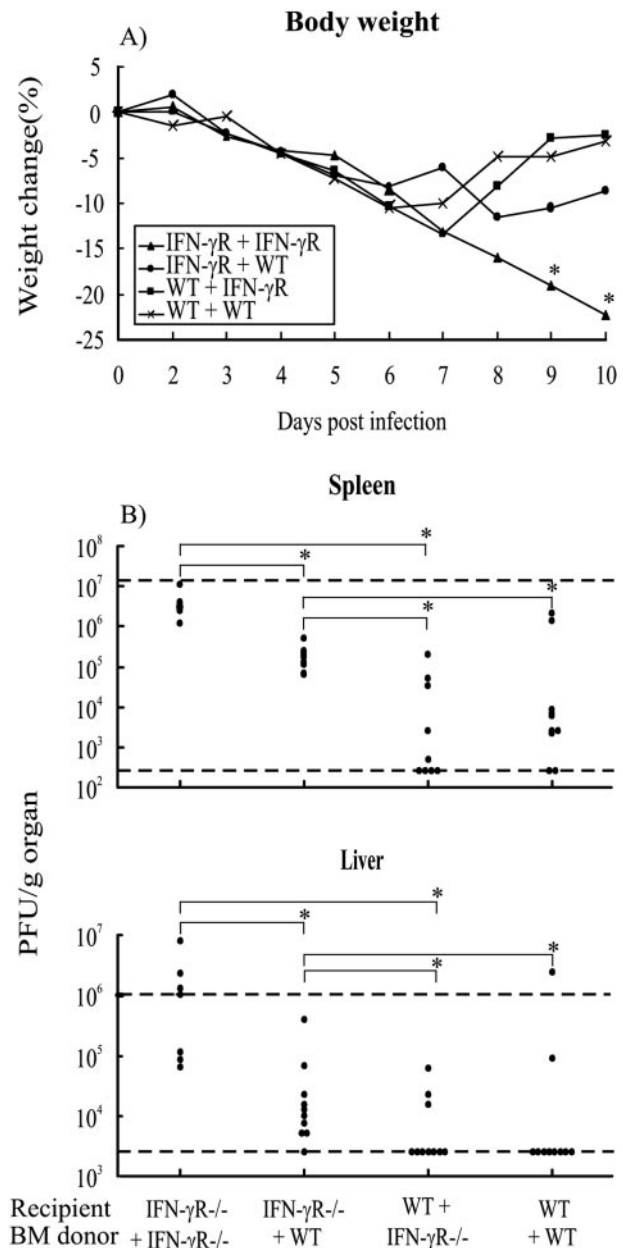


FIG. 3. Severe wasting disease and complete impairment of virus clearance in chimeric mice lacking IFN- γ R expression in the hematopoietic and parenchymal compartments. Syngeneic and allogeneic chimeras were constructed from IFN- γ R^{-/-} and WT mice. Lethally irradiated (900 rads) mice received 20×10^6 bone marrow cells (BM) intravenously. Eight weeks later the chimeras were given 3×10^6 cells from TCR-tg mice and then challenged with 10^3 LD₅₀ LCMV Traub i.v. 1 day later. The mice (10 mice/group) were weighed daily until day 10 postinfection; medians of the changes in body weight are presented (A). Spleens and livers were harvested day 10 postinfection, and the virus titers were determined; points represent individual mice (B). The lower dashed lines indicate the detection limit, and the upper dashed lines indicate the medians of organ virus titers in unmanipulated IFN- γ R^{-/-} mice challenged with 10^3 LD₅₀ of LCMV Traub. *, statistically significant differences at $P < 0.05$ (Mann-Whitney U test).

cells to control the infection. If, however, no such restriction is obtained, indirect antiviral effects such as that mediated through activated macrophages may become more important.

These findings may contribute to a better understanding of the mechanisms underlying T-cell-mediated control of systemic infections with noncytolytic viruses such as hepatitis B and human immunodeficiency virus, in which a shift in the T-cell response may intensify the induced pathology.

This study was supported in part by the Novo-Nordisk foundation and the Lundbeck Foundation. P.H. was the recipient of a scholarship from Novo Nordisk, and C.B. was the recipient of a postdoctoral fellowship from the Danish Medical Research Council.

REFERENCES

- Bartholdy, C., J. P. Christensen, D. Wodarz, and A. R. Thomsen. 2000. Persistent virus infection despite chronic cytotoxic T-lymphocyte activation in gamma interferon-deficient mice infected with lymphocytic choriomeningitis virus. *J. Virol.* **74**:10304–10311.
- Battegay, M., S. Cooper, A. Althage, J. Banziger, H. Hengartner, and R. M. Zinkernagel. 1991. Quantification of lymphocytic choriomeningitis virus with an immunological focus assay in 24- or 96-well plates. *J. Virol. Methods* **33**:191–198.
- Blattman, J. N., R. Antia, D. J. Sourdive, X. Wang, S. M. Kaech, K. Murali-Krishna, J. D. Altman, and R. Ahmed. 2002. Estimating the precursor frequency of naive antigen-specific CD8 T cells. *J. Exp. Med.* **195**:657–664.
- Christensen, J. E., J. P. Christensen, N. N. Kristensen, N. J. Hansen, A. Stryhn, and A. R. Thomsen. 2002. Role of CD28 co-stimulation in generation and maintenance of virus-specific T cells. *Int. Immunol.* **14**:701–711.
- Christensen, J. E., D. Wodarz, J. P. Christensen, and A. R. Thomsen. 2004. Perforin and IFN-gamma do not significantly regulate the virus-specific CD8+ T cell response in the absence of antiviral effector activity. *Eur. J. Immunol.* **34**:1389–1394.
- Dalton, D. K., S. Pitts-Meek, S. Keshav, I. S. Figari, A. Bradley, and T. A. Stewart. 1993. Multiple defects of immune cell function in mice with disrupted interferon-gamma genes. *Science* **259**:1739–1742.
- Fiette, L., C. Aubert, U. Muller, S. Huang, M. Aguet, M. Brahic, and J. F. Bureau. 1995. Theiler's virus infection of 129Sv mice that lack the interferon alpha/beta or interferon gamma receptors. *J. Exp. Med.* **181**:2069–2076.
- Graham, M. B., D. K. Dalton, D. Giltinan, V. L. Braciale, T. A. Stewart, and T. J. Braciale. 1993. Response to influenza infection in mice with a targeted disruption in the interferon gamma gene. *J. Exp. Med.* **178**:1725–1732.
- Huang, S., W. Hendriks, A. Althage, S. Hemmi, H. Bluethmann, R. Kamijo, J. Vilcek, R. M. Zinkernagel, and M. Aguet. 1993. Immune response in mice that lack the interferon-gamma receptor. *Science* **259**:1742–1745.
- Kagi, D., and H. Hengartner. 1996. Different roles for cytotoxic T cells in the control of infections with cytopathic versus noncytopathic viruses. *Curr. Opin. Immunol.* **8**:472–477.
- Kagi, D., B. Ledermann, K. Burki, P. Seiler, B. Odermatt, K. J. Olsen, E. R. Podack, R. M. Zinkernagel, and H. Hengartner. 1994. Cytotoxicity mediated by T cells and natural killer cells is greatly impaired in perforin-deficient mice. *Nature* **369**:31–37.
- Kristensen, N. N., J. P. Christensen, and A. R. Thomsen. 2002. High numbers of IL-2-producing CD8+ T cells during viral infection: correlation with stable memory development. *J. Gen. Virol.* **83**:2123–2133.
- Kundig, T. M., H. Hengartner, and R. M. Zinkernagel. 1993. T cell-dependent IFN-gamma exerts an antiviral effect in the central nervous system but not in peripheral solid organs. *J. Immunol.* **150**:2316–2321.
- Lućin, P., I. Pavić, B. Polić, S. Jonjić, and U. H. Koszinowski. 1992. Gamma interferon-dependent clearance of cytomegalovirus infection in salivary glands. *J. Virol.* **66**:1977–1984.
- Mo, X. Y., R. A. Tripp, M. Y. Sangster, and P. C. Doherty. 1997. The cytotoxic T-lymphocyte response to Sendai virus is unimpaired in the absence of gamma interferon. *J. Virol.* **71**:1906–1910.
- Muller, U., U. Steinhoff, L. F. Reis, S. Hemmi, J. Pavlovic, R. M. Zinkernagel, and M. Aguet. 1994. Functional role of type I and type II interferons in antiviral defense. *Science* **264**:1918–1921.
- Nansen, A., T. Jensen, J. P. Christensen, S. O. Andreassen, C. Ropke, O. Marker, and A. R. Thomsen. 1999. Compromised virus control and augmented perforin-mediated immunopathology in IFN-gamma-deficient mice infected with lymphocytic choriomeningitis virus. *J. Immunol.* **163**:6114–6122.
- Ou, R., S. Zhou, L. Huang, and D. Moskophidis. 2001. Critical role for alpha/beta and gamma interferons in persistence of lymphocytic choriomeningitis virus by clonal exhaustion of cytotoxic T cells. *J. Virol.* **75**:8407–8423.
- Pircher, H., D. Moskophidis, U. Rohrer, K. Burki, H. Hengartner, and R. M. Zinkernagel. 1990. Viral escape by selection of cytotoxic T cell-resistant virus variants in vivo. *Nature* **346**:629–633.
- Ramsay, A. J., J. Ruby, and I. A. Ramshaw. 1993. A case for cytokines as effector molecules in the resolution of virus infection. *Immunol. Today* **14**:155–157.
- Ramshaw, I. A., A. J. Ramsay, G. Karupiah, M. S. Rolph, S. Mahalingam, and J. C. Ruby. 1997. Cytokines and immunity to viral infections. *Immunol. Rev.* **159**:119–135.
- Sarawar, S. R., R. D. Cardin, J. W. Brooks, M. Mehrpooya, A. M. Hamilton-Easton, X. Y. Mo, and P. C. Doherty. 1997. Gamma interferon is not essential for recovery from acute infection with murine gammaherpesvirus 68. *J. Virol.* **71**:3916–3921.
- Schijns, V. E., C. M. Wierda, M. van Hoeij, and M. C. Horzinek. 1996. Exacerbated viral hepatitis in IFN-gamma receptor-deficient mice is not suppressed by IL-12. *J. Immunol.* **157**:815–821.
- Smith, P. M., R. M. Wolcott, R. Chervenak, and S. R. Jennings. 1994. Control of acute cutaneous herpes simplex virus infection: T cell-mediated viral clearance is dependent upon interferon-gamma (IFN-gamma). *Virol. J.* **202**:76–88.
- Tishon, A., H. Lewicki, G. Rall, M. Von Herrath, and M. B. Oldstone. 1995. An essential role for type 1 interferon-gamma in terminating persistent viral infection. *Virology* **212**:244–250.
- Topham, D. J., R. A. Tripp, and P. C. Doherty. 1997. CD8+ T cells clear influenza virus by perforin or Fas-dependent processes. *J. Immunol.* **159**:5197–5200.
- Utermohlen, O., A. Dangel, A. Tárnok, and F. Lehmann-Grube. 1996. Modulation by gamma interferon of antiviral cell-mediated immune responses in vivo. *J. Virol.* **70**:1521–1526.
- van den Broek, M. F., U. Muller, S. Huang, R. M. Zinkernagel, and M. Aguet. 1995. Immune defence in mice lacking type I and/or type II interferon receptors. *Immunol. Rev.* **148**:5–18.
- Von Herrath, M. G., B. Coon, and M. B. Oldstone. 1997. Low-affinity cytotoxic T-lymphocytes require IFN-gamma to clear an acute viral infection. *Virology* **229**:349–359.
- Walsh, C. M., M. Matloubian, C. C. Liu, R. Ueda, C. G. Kurahara, J. L. Christensen, M. T. Huang, J. D. Young, R. Ahmed, and W. R. Clark. 1994. Immune function in mice lacking the perforin gene. *Proc. Natl. Acad. Sci. USA* **91**:10854–10858.
- Wherry, E. J., J. N. Blattman, K. Murali-Krishna, R. van der Most, and R. Ahmed. 2003. Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment. *J. Virol.* **77**:4911–4927.
- Wille, A., A. Gessner, H. Lother, and F. Lehmann-Grube. 1989. Mechanism of recovery from acute virus infection. VIII. Treatment of lymphocytic choriomeningitis virus-infected mice with anti-interferon-gamma monoclonal antibody blocks generation of virus-specific cytotoxic T lymphocytes and virus elimination. *Eur. J. Immunol.* **19**:1283–1288.